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Removal of parasitic particles, protozoa cysts, and thermotolerant coliforms in the integrated aeration lagoon, case study: Iran

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ABSTRACT

The aim of this study is to determine the efficiency of integrated aerated lagoon (with sludge recycling) on removal of parasite eggs, protozoan cysts, and thermotolerant coliforms from wastewater of Marivan city in Iran. This descriptive–analytical study was carried out during 6 months and samples were collected at weekly intervals from influent and effluent. The counting of parasite eggs and protozoan cysts carried out using Bailenger method. Thermotolerant coliforms are determined according to standard method. Engelberg index was also surveyed. Statistical analytic was accomplished by SPSS (Kolmogrov Smirnov test and Mann-Whitney u test). No nematode, *Trichuris trichiura* egg, *Ascaris lumbricoides* egg, and *Entamoeba coli* cyst were detected in the effluents of aerated lagoon. The efficiency of process in removing parasite eggs and protozoan cysts was >99%. Aerated lagoon was capable of thermotolerant coliform removal from 88.1% (warm weather) until 80.37% (cold weather). The results of this study indicate that aerated lagoon process can achieve good microbial removal in proper condition. Irrigation with lagoon effluent is saved by parasite not thermotolerant coliform. The outlet effluent quality of in terms of nematode eggs is consistent with Engelberg index. Any correlation between the thermotolerant coliform, protozoa cyst, and parasite egg observed.

Keywords: Engelberg index; Protozoan cysts; Thermotolerant coliform; Integrated aeration lagoon; Iran

1. Introduction

Due to the increasing population and the need for more water resources, reuse of municipal wastewater in agriculture and irrigation, especially in areas where there is a shortage of water, it can be very significant as an appropriate economic option [1]. The reuse of treated wastewater in the agricultural has many advantages, including nutrient supply, reducing fertilizer use, and the cost of treatment, reducing environmental impacts due to the disposal of waste water into water resources and so on [2–4]. Of course, the complications due to the lack of adequate treatment of sewage and the use of untreated wastewater on the environment should also be taken seriously, especially when it needs to be used in the agricultural. The presence of various microbial pollutants in the wastewater leads to contamination of water resources [5], soil [6], and agricultural products [7] and its health hazards affect human health. This will be more important when wastewater is used to irrigate the

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green space and food products, including raw vegetables and planting fruits. In most developing countries, mortality due to water pollution, especially child mortality, is a public health concern. Transmission of the disease through microorganisms in the wastewater is very important [8]. In this regard, the World Health Organization's guidelines for the microbiology quality of treated wastewater for irrigation has been developed based on available evidence of contagious diseases and their severity. Hence, the most important pathogens are intestinal nematodes and fecal bacteria [9]. Therefore, comprehensive management of wastewater treatment as well as monitoring of the quality of wastewater that will be reused will be essential. Wastewater contamination with fecal coliforms, eggs of parasites and protozoan cysts are among the most important sources of infectious diseases [10]. Some conditions, including climatic and geographical conditions, culture and health habits of the people, can be effective in determining the prevalence of parasite infection in the community [11]. Reports of parasitic and bacterial contamination in reuse of wastewater and its effects on the health of millions of people such as typhoid, diarrhea, tetanus, meningitis, and hepatitis have been reported [8]. Coliform bacteria are the most common group of indigenous microorganisms in determining the rate of microbial contamination. Therefore, the criteria for assessing the quality of the microbial wastewater are presented in most standards and guidelines based on the frequency of this group of microorganisms [12]. Reducing the risks of wastewater to health and the environment requires the use of an efficient treatment process. Different methods have been reported to reduce bacterial and parasitical contamination including UASB [13], soil layered process, sand filters [14], and pressure filters [15]. Aeration lagoons are one kind of wastewater treatment process that convert waste materials using artificial aeration in a pond and provide conditions for the growth and propagation of microorganisms [16]. The efficiency of aeration lagoons in reducing pathogens is unstable and is affected by environmental factors, qualitative characteristics of wastewater, and operating conditions [17]. The mechanism of removal of parasite and protozoan cysts in different purification process is different [18,19]. Although their density above the water resulting in sedimentation, filtration, trapping in sludge, etc., are among the main mechanisms of removal. In the aeration lagoon, the sedimentation role is much more prominent than other parameters [20].

Considering the fact that so far studies on the role of natural-purified wastewater treatment on the real scale for the removal of eggs of parasite, protozoan cysts, and bacteria in Iran's climate and especially in the mountainous climate of western Iran is low. Therefore, the aim of this study was to reduce egg parasites, cysts, and fecal coliform bacteria in real-scale using natural purification process (aerated lagoon) in Marivan, Iran. We have also investigated the relation between egg parasite and protozoan cysts with indicator bacteria. Treated wastewater was investigated for Engelberg index for reuse in agriculture.

2. Materials and methods

The experimental study was carried out from July to December 2015 on influent and effluent of Marivan wastewater treatment plant (MWTP). All of the samples (No. 92) were collected, stored, and preserved in polyethylene containers and analysis were performed based on standard methods procedures for water and wastewater examinations [21]. Volume of samples for influent and effluent were 100 mL and 10 L, respectively. Parasitological experiments were done at by modified Bailenger method with McMaster counting slides [22]. Remaining sediment after sample sedimentation process centrifuged at 1,000 g for 15 min. The sediment of centrifuge tubes transfers to one centrifuge tube and centrifuge it again at 1,000 g for 15 min. Then the pallet was suspended in equal volume of stokes buffer (pH = 4.5) and doubled volume of acetyl acetate were added. The sample was mixed entirely by stirring and then was centrifuged at 1,000 g for 15 min. Two layers were taken place in all centrifuge tubes and removed (upper black and middle layer). Then, sediment was suspended in zinc sulfate (density 33%) and mixed. This solution (precipitate + zinc sulfate) was considered as adequate volume of final product. Quickly, 0.3 mL was transferred to slide microscopic counting with a magnification of 100 and 40. The number of cysts and parasite eggs was identified and counted. We used the Eq. (1) to calculate the number of cysts and eggs in samples. Total number of thermotolerant coliforms obtained according to the standard method [22].

$$N = \frac{AX}{PV} \tag{1}$$

where N: the average number of eggs or cysts counted in three slides under microscopic observation, X: the final volume of product (mL), P: the volume put on the McMaster slide (0.3 mL), and V: the original sample volume (mL).

Statistical analysis was carried out using SPSS 16 with a level of significance of 0.05. The statistic index was used to determine the statistical parameters such as standard deviation and microorganism reduction efficiency.

3. Result and discussion

3.1. Microbial condition in MWTP

Parasitic contamination changes in wastewater entering the MWTP during the warm and cold weather are shown in Tables 1 and 2, respectively. According to our obtained results, it can be concluded that the wastewater entering the MWTP in the warm and cold weathers has the highest and lowest levels of pollution, respectively. *Entamoeba coli* and *Giardia lamblia* and protozoan cyst were observed in raw wastewater. *Ascaris lumbricoides*, *Trichuris trichiura*, and *Hymenolepis nana* were identified as helminth eggs. However, the seeds of *H. nana* and *T. trichiura* were not observed during the warm weather, but in the cold weather, their number was found to be 6.57 and 1.53, respectively. The effectiveness of aerobic lagoon in the removal of protozoan cysts, nematode eggs, entamoeba cysts, giardia cysts, and A. lumbricoides reached 100% and the effluent was safe from this view. Based on the Mann-Whitney U test ($\alpha = 0.05$), it was found that giardia cyst, *E. coli* cyst, and *A. lumbricoides* egg remained during the warm weather but in other cases, no significant difference was observed. Totally, more than 99% of parasite egg in influent (among nematodes, the eggs of the *A. lumbricoides*

Table 1
Mean amount of different parameters in raw wastewater and effluent from aerated lagoon of Marivan warm

Sample	Influent	Effluent
Protozoan cysts	45.04 ± 8.16	0.49 ± 0.06
Nematode egg	24.1 ± 3.86	0
Total parasite egg	23.9 ± 4.74	0.11 ± 0.06
Antamoba coli cyst	17.32 ± 0.87	0
Giardia cysts	27.71 ± 4.47	0.51 ± 0.02
Hymenolepis nana egg	0	0
Trichories trichoria egg	0	0
Ascaris lumbricoides egg	28.34 ± 3.77	0
Thermotolerant coliform	26.12×10^5	31.08×10^4

Table 2
Mean amount of different parameters in raw wastewater and effluent from aerated lagoon of Marivan cold

Sample	Influent	Effluent
Protozoan cysts	17.02 ± 2.76	0
Nematode egg	22.54 ± 3.86	0
Total parasite egg	31.25 ± 9.74	0.27 ± 0.04
Antamoba coli cyst	4.52 ± 0.57	0
Giardia cysts	12.5 ± 1.47	0
Hymenolepis nana egg	6.52 ± 1.57	0.35 ± 0.06
Trichories trichoria egg	0.11 ± 0.07	0
Ascaris lumbricoides egg	24.71 ± 7.65	0
Thermotolerant coliform	54.21×10^5	10.63×10^5

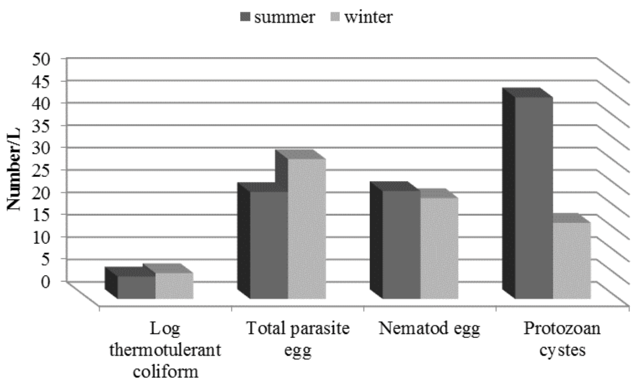

Fig. 1. Average microbial contamination in Marivan raw wastewater in warm and cold conditions.

and the *Trichiurus trikora*) eggs was observed. Among the probable protozoans in raw wastewater, only giardia cysts and *E. coli* could be observed. It can be concluded that the logarithm of the thermal infiltration of raw wastewater in the warm weather are less than cold weather. Maximum process efficiency during the warm and cold weather is found to 88.1% and 80.77%, respectively.

Regarding the proper environmental conditions for growth and replication of microorganisms, the parasitic contamination in raw wastewater produced during the warm weather was higher than the one in cold weather. The results of our experiments indicate that the highest number of eggs in the raw wastewater in warm and cold weather are belongs to protozoa cyst *Ascaris lambriocides*, respectively. This is due to their high resistance to other eggs of the parasites, including *H. nana* and *T. trikora* against adverse environmental conditions. We have also shown that the average number of eggs of nematode parasites in the lagoon effluent was less than one per liters in the warm and cold weathers, and there is no problem with irrigation according to the Engelberg index [23]. The Engelberg Quality Guideline has calculated the average of the live nematode eggs in the treated wastewater as a number per liter for both kind of restricted and unrestricted irrigations. Furthermore, the results of the present work show that the aerated lagoon of Marivan has been successful. The results of our study contradict with the report of Ghasemi and Danesh [24] in Mashhad. So that without effective disinfection, aeration lagoons were not able to meet the microbiological standards of wastewater use in agriculture [24]. Our study showed that protozoa cysts are more intense than giardia and *E. coli* cysts in wastewater. Parasitic contamination has always been reported in Iran [22,25,26]. The average number of eggs in the Marivan wastewater is lower than the Peruvian wastewater (194 ± 79) [13]. On the other hand, the results reported by Sossou et al. [27] showed that during 60 d at alkaline pH and low temperatures, it cannot completely induce bacterial degradation while Salmonella after 30. Entamoeba cysts were present at higher concentrations (854 g/g) of *A. lumbricoides* (204 g/g). Entamoeba cysts were also more resistant than ascaris eggs. Due to the high resistance of ascaris eggs and entamoeba cysts they can be considered as good indicators [28]. *E. coli* and giardia are commonly present in purification process which are aligned with the results in countries such as Italy [29], Malaysia [30], and France [31]. In the US study, the average oocyst concentration in the outlet was including 2% entamoeba coli and giardia [29]. The highest survival time was reported for *A. lumbricoides*, *T. trichiura*, and *H. nana* for 3 years [32], 2 years [33], and 7 months [34], respectively. Shingare et al. [35] who used *T. latifolia* to remove intestinal parasites from wetland reported 100% efficiency in the removal of fecal coliform. The average of nematode eggs found in Brazil [36] and Peru [13] was higher than those of study. The discrepancy in various studies can be attributed to differences in wastewater collection system and runoff flows, the combination and population density, the density of industrial areas in the city, the sampling method, and the validity of the results. The performance of aerated lagoons depends on weather conditions, especially temperature which determines the dominant species of microorganisms in the lagoon. Due to the high dependence of this type of treatment method on temperature changes in the cold season as a result of reduced microbial activity the presence of microbes was less observed however there was no significant difference in the efficiency of the process in the cold and warm weather. The parasitic contamination may be due to the migration of people from suburban areas to lower levels of health into the city and the lack of sanitation and welfare facilities [22]. Therefore, in order to reduce the parasitic infection, it has been tried to improve the social, economic and health conditions of the people by improving the systems for collecting and treating wastewater. Investigation of the number of thermotolerant coliforms in the wastewater entering the warm and cold seasons showed that their populations reach the lagoon at 26.12×10^5 and 54.21×10^5 colonies. It shows that sewage is in the strong sewage category in terms of severity of pollution. According to the Engelberg index, the geometric mean of fecal coliforms for unlimited irrigations is allowed up to 1,000 per 100 mL [37]. In the study, despite the high residence time in lagoon, fecal coliforms did not decrease to the standard of the World Health Organization for unlimited irrigation. Therefore, it is necessary to pay special attention to the climate conditions of the area in the design of the treatment plant and provide sufficient time to meet the Engelberg index in the lagoon effluent. Because the raw wastewater is in the severity of pollution in the strong wastewater group, the removal of 5 log units in the MWTP would be essential. Raw wastewater with a high degree of contamination requires a higher percentage of removal of coliform than medium-sized wastewater sludge.

3.2. Relationship between cysts and microorganisms Indicator

Spearman's nonparametric random correlation test was used to evaluate the relation between parasitic pollution and microbial indices. However, this relationship could not be significant. The results of our study are consistent with the results of Harwood et al. [38], Brianceco et al. [29], and Fu et al. [39]. In the study of Brianceco, the association between fecal coliform and giardia cyst was not significant. This lack of correlation may be due to differences in their microbial behavior based on the type of treatment process, applying conditions, water flow, environmental, and weather conditions.

4. Conclusion

It can be concluded that aerated lagoon in this study is unable to meet the quality standards of wastewater treatment in irrigated agricultural products from fecal coliform. However, with a reduction of 80.37% of fecal coliform in the cold weather and 88.1% in the warm weather, it can reduce the amount of chlorine used for disinfection. As it can be seen, the highest and lowest removal percentage of was observed in warm and cold weathers, respectively, mainly due to changes in climatic conditions.

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